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Interactions of aristololactam-β-D-glucoside with deoxyribonucleic acids : spectroscopic, viscometric and thermodynamic aspects of binding

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Abstract : The binding of aristololactam-β-D-glucoside to DNA is characterized by hypochromism and bathochromism in the absorption band, quenching of the fluorescence intensity, increase in the positive and negative ellipticity of DNA, enhancement of thermal transition temperature, sign and magnitude of thermodynamic parameters, increase of the contour length of sonicated rod-like DNA and induction of the unwinding-rewinding process of covalently closed superhelical DNA. Intrinsic binding constants of four double-helical DNA species from different sources vary by a factor of approximately 2.5 and are related to the gross base composition being larger with GC rich DNA than with AT rich DNA. Temperature dependence of the binding constants are used to estimate the thermodynamic parameters involved in the interaction of the alkaloid with four natural DNAs. It is observed that the alkaloid binding to each DNA is exothermic process over the entire range of temperature. The enthalpy and entropy changes compensate one another to produce a relatively small Gibbs' free energy change. The entropy change is also dependent on the base composition of DNA. On the basis of these observations, it is concluded that aristololactam-β-D-glucoside binds to natural DNA by a mechanism of intercalation and its complexing has a preference for GC rich natural DNA. The possible molecular contribution to the enthalpy and entropy changes of the aristololactam-β-D-glucoside-DNA complex is discussed.

Keywords : Aristololactam-β-D-glucoside, DNA, alkaloid-DNA interaction.

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1. Introduction

The biological activities of many compounds are presumed to be due to their interaction with DNA, interfering with its expression in the cells (Waring 1981a, Neidle and Waring 1983). The physical and molecular basis of interaction of small molecules to DNA has been a subject of extensive study in the recent past (Zimmer and Wahnert 1986, Wilson 1989).

Aristololactam-β-D-glucoside (ADG) or 6-β-D-glucopyranosyl-8-methoxy benzo (f)-1,3-benzodioxolo (6,5,4-cd) indol-5(6H)-one (Figure 1) is a broad spectrum biologically active compound that belongs to the group of Aristolochia alkaloids

which have attracted recent attention for their prospective clinical and pharmacological uses (for review see Chen and Zhu 1987). It has pH dependent spectral

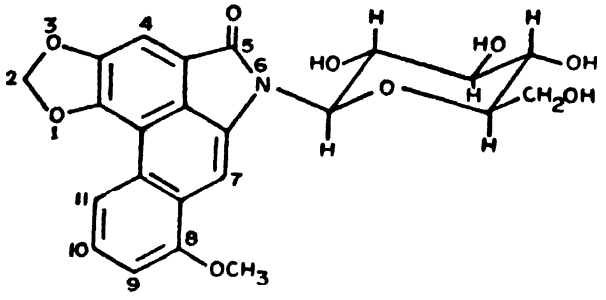

Figure 1. Chemical structure of aristololactam-β-D-glucoside.

changes from the standpoint of its absorption and fluorescence spectra (Chakraborty *et al* 1989a). The mechanism of action, particularly at the molecular level of this alkaloid was not known and has been under investigation in our laboratory. Recently, it has been shown that it forms a complex with calf thymus DNA (Chakraborty *et al* 1989b, 1990), but the molecular nature of its interaction with DNA has remained obscure.

In order to gain some insight into the molecular nature of its specificity towards DNA of varying base composition, a series of physicochemical measurements on the interaction of aristololactam-β-D-glucoside with several natural DNAs of varying base composition has been carried out in this study.

2. Materials and methods

Clostridium perfringes (CP) DNA (type XII, 30 mole % GC), calf thymus (CT) DNA (type I, 42 mole % GC), *Escherichia coli* (EC), strain B DNA (type VII, 50 mole % GC), *Micrococcus lysodeikticus* (ML) DNA (type XI, 72 mole % GC), covalently closed superhelical Col EI plasmid (CCS Col EI) DNA (53 mole % GC) and dimethyl sulphoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis MO, USA). All DNA were used without further purification. Their nativeness and purity were tested by spectrophotometry and circular dichroic (CD) spectra (Maiti and Chaudhuri 1981, Maiti *et al* 1982, 1984, Maiti and Nandi 1986, 1987a, b, Debnath *et al* 1989). All polymer concentrations were determined spectrophotometrically using the molar extinction coefficients reported earlier (Maiti and Nandi 1987a, Debnath *et al* 1989).

Aristololactam-β-D-glucoside (ADG) was extracted from *Aristolochia indica* and was crystallized from ethanol. Its purity was checked as described previously (Chakraborty *et al* 1989a). The alkaloid concentration was determined spectrophotometrically using molar extinction coefficient (ϵ) of 10930 $M^{-1}cm^{-1}$ at 398 nm in dimethyl sulphoxide (DMSO). Deionized glass distilled water and analytical grade reagents were used throughout. Except Tm measurements, all DNA binding

experiments were performed in a BPES-DMSO buffer containing 1.5 mM Na_2HPO_4, 0.5 mM NaH_2PO_4, 0.25 mM EDTA, 240 mM DMSO, pH 7.0 ± 0.05 and different Na^+ molarity obtained by addition of required volumes of sodium chloride solution from a known concentrated stock. Tm measurements were conducted in BPE-DMSO buffer, pH 6.9 ± 0.05 containing 1.5 mM Na_2HPO_4, 0.5 mM NaH_2PO_4, 0.25 mM EDTA and 240 mM DMSO. The properties of DNA remained unaltered in these buffers as revealed by absorbance, Tm, viscosity and circular dichroic data.

Absorption spectra of ADG mixed with or without DNA were obtained at 25°C using the Shimadzu UV-260 automatic recording spectrophotometer (Shimadzu Corporation, Japan) against an appropriately prepared reference sample in a 1 cm quartz cuvette. Spectrophotometric titrations of the alkaloid-DNA mixtures were carried out at 25°C generally following the method earlier (Maiti *et al* 1982, 1984).

Fluorescence measurements were recorded at 25°C in a Farrand System 3 Spectrofluorimeter (Farrand Optical Co., U.S.A.), using a 1 cm quartz cuvette. Emission spectra of the alkaloid alone and in the presence of increasing concentration of DNA were measured. The concentrations of free and bound alkaloid were calculated from the fluorescence titrations as previously described (Maiti *et al* 1983, 1984, Nandi *et al* 1985). Uncorrected fluorescence spectrum was recorded. The alkaloid obeyed Beer's law in the concentration range used in this study.

To estimate the thermodynamic parameters, the binding studies were done at 15°, 25° and 40°C from the measurements of absorption spectrophotometry and the binding constants were determined either from a complete titration at the given temperature or by increasing the temperature of a sample containing a fixed ratio of alkaloid/DNA as described in (Chaires 1985, Chakraborty *et al* 1990).

Thermal melting profiles of DNA and alkaloid-DNA complex were determined using the Shimadzu UV-260 spectrophotometer, equipped with a thermoelectric cell temperature controller S-260/SPR-5 and temperature programmer KPC-5 at the rate of 1°C per min in 1 cm quartz cuvette. CD spectra were recorded at 25°C in a cylindrical cell of 1 cm path length on a Jasco J-20A spectropolarimeter (Japan Specctroscopic Ltd., Japan) with a data processor attachment model J-DPY as stated in (Maiti 1986, Maiti and Nandi 1987a, b, Maiti *et al* 1984, 1985). CD results were derived from the mean values of at least three determinations and were expressed as molar ellipticity [θ] in units of degree cm^2/d mol.

For viscometric studies, linear duplex DNA was sonicated in a Labsonic 2000 (B. Braun, Swiss) sonicator with needle probe as described previously (Maiti *et al* 1982, 1984, Nandi and Maiti 1985, Nandi *et al* 1990). The estimated molecular weight of DNA was found to be of about 3.5×10^5 (Nandi and Maiti 1985). The measurement of viscosity of linear sonicated natural DNA (300 μM); CCS Col EI DNA (150 μM) and their complexes were done with a type 75 Cannon Manning Semimicro Viscometer using the method described previously (Maiti *et al*

1982, 1985, Debnath et *al* 1989). Temperature was maintained at 25 ± 0.1°C in a Cannon constant temperature bath model MI (Cannon Instrument Co., U.S.A.).

3. Results

3.1. Absorption spectra of the ADG-DNA complexes :

The ultraviolet visible absorption spectrum of ADG is characterised by four absorption maxima centred around 259, 290, 330 and 398 nm respectively (Chakraborty et *al* 1989a). In presence of increasing concentrations of DNA, bathochromic and hypochromic effects were observed in all the absorption bands, but the effects were more pronounced in the 398 nm band. The typical changes observed in the absorption spectrum of the alkaloid in presence of CT DNA are illustrated in Figure 2. The series of spectra exhibited an isosbestic point at

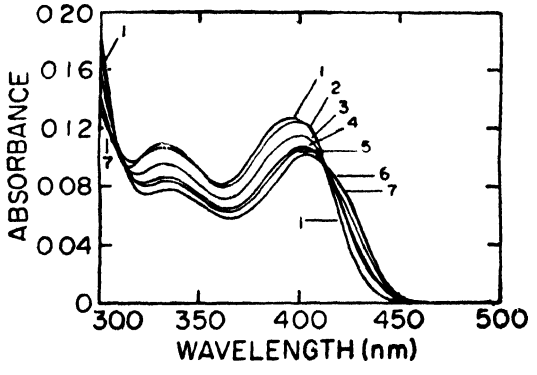

Figure 2. Effect of CT DNA on the absorption spectrum of ADG in BPES-DMSO buffer, pH 7.0. Curves (1-7) denote 15 μM of ADG treated with 0, 26.9, 77.1, 126.12, 180.5, 275.2, 325.5 μM of DNA respectively.

416 nm, indicating an equilibrium between the free and bound ligand. A maximum red shift of 12 nm of 398 nm band was observed at saturation ($P/D > 16$). Similar features in the spectral changes were observed with other natural DNAs.

3.2. Evaluation of binding parameters :

The results of absorption titration were expressed in the form of Scatchard plot (Scatchard 1949) r vs r/C, where r is the moles of bound ligand/moles of nucleotide, C is the molar concentration of free ligand. For concave binding isotherm, experimental data were fitted to the excluded-site model (Crothers 1968, McGhee and von Hippel 1974).

$$r/C = K(1-nr)[(1-nr)/(1-(n-1)r)]^{n-} \quad (1)$$

where K is the intrinsic binding constant to an isolated site and n is the exclusion parameter as described previously (Nandi et *al* 1985, Choudhuri et *al* 1983, Nandi and Maiti 1985). The best fit values for K and n were analysed (data not shown).

The quantitative data of the binding parameters presented in Table 1, indicates that the values for the GC-rich ML DNA differ from that of AT-rich CP DNA, revealing that the higher GC content DNA binds with ADG more efficiently. The dependence of interaction of the alkaloid on base composition of DNA can be

Table 1. Binding parameters for the interaction of ADG with various natural DNAs[a].

Polymer	GC mole %	K ($\times 10^5$ M^{-1})	n	α[b]
CP DNA	30	1.7 ± 0.08	7.1 ± 0.1	—
CT DNA	42	2.6 ± 0.2	6.1 ± 0.15	1.733
EC DNA	50	2.8 ± 0.15	6.1 ± 0.2	1.80
ML DNA	72	3.8 ± 0.2	5.9 ± 0.15	2.53

[a]Five determination each. [b]The ratio is based on the equation $\alpha = K_{DNA}/K_{CP\ DNA}$ calculated from the results as described in (Nandi and Maiti 1985).

examined in terms of α-values obtained by excluded site analysis described earlier (Nandi and Maiti 1985). The α values of greater than 1.63 (Table 1) would, of course, denote GC specificity in binding.

3.3. *Thermodynamic parameters :*

The thermodynamic study of the interaction between ADG and DNA has been carried out, based on the temperature-dependence of the binding constant using the neighbour exclusion model to fit the experimental data. The Gibbs free energy change was determined from the binding constant according to the relationship

$$\Delta G = -RT \ln K \tag{2}$$

The binding enthalpy ΔH was determined from the plots of the temperature dependence of the binding constant according to the van't Hoff relationship

$$\delta \ln K/\delta(1/T) = -\Delta H/R \tag{3}$$

The entropy was estimated from the Gibbs free energy and the enthalpy as

$$\Delta S = -(\Delta G - \Delta H)/T \tag{4}$$

The effect of progressive increment in the concentration of DNA on the absorption spectrum of ADG was studied at three different temperatures in BPES-DMSO buffers with 20 mM sodium ion at pH 7.0. The spectrophotometric measurements in buffer of a particular sodium molarity were obtained at 15°, 25° and 40°C and presented in Figure 3. The spectral changes involve essentially a red shift and hypo-chromicity in complexes until saturation is reached. However, hypochromicity for a particular ADG/DNA ratio decreases with increase in temperature.

The spectrophotometric titration data for the complex at various salt molarities and temperatures were analysed and calculated (Scatchard plots not shown).

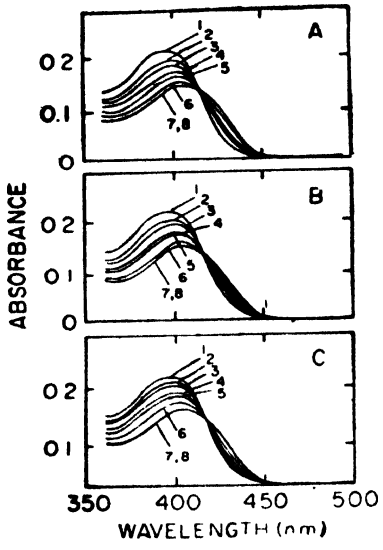

Figure 3. Influence of CT DNA on the absorption spectrum of ADG in BPES-DMSO buffer, pH 7.0. Curves (1-8) denote 20.1 μM of ADG treated with 0, 75.5, 132.5, 227.0, 302.5, 576.2, 962.8, 1298.2 μM of DNA respectively at 15°C (A), 25°C (B) and 40°C (C).

Table 2. Thermodynamic parameters for the interaction of ADG with natural DNAs[a].

Polymer	Temperature (°C)	K ($\times 10^5$ M^{-1})	$-\Delta G$ (25°C) (KCal/mol)	$-\Delta H$ (KCal/mol)	$-\Delta S$ (25°C) (Cal/degree/mol)
CP DNA	15	3.2 ± 0.3	7.17 ± 0.07	10.9 ± 0.4	12.5 ± 1.5
	25	1.7 ± 0.2			
	40	0.72 ± 0.04			
CT DNA	15	5.8 ± 0.3	7 43 ± 0.04	13.8 ± 0.2	21.5 ± 0.6
	25	2.6 ± 0 2			
	40	0.8 ± 0.04			
EC DNA	15	5.8 ± 0.02	7.47 ± 0.04	14.04 ± 0.6	22.19 ± 1.7
	25	2.8 ± 0.2			
	40	0.82 ± 0.05			
ML DNA	15	9.7 ± 0.4	7.65 ± 0.03	15.5 ± 0.5	26.24 ± 1.61
	25	3.8 ± 0.2			
	40	1.2 ± 0.1			

[a]Five determinations each.

The results of thermodynamic parameters are presented in Table 2. It can be seen from the Table 2 that the values of $\Delta H = -13.8$ KCal/mole and $\Delta S = -21.5$ Cal/degree/mole at 25°C are of same sign as and a similar magnitude to values obtained for other intercalators (Chaires 1985, Barcelo *et al* 1988).

3.4. Fluorescence spectra of the ADG-DNA complexes :

Earlier it has been reported (Chakraborty *et al* 1989a) that ADG had a fluorescence spectrum with emission band at 485 nm in BPES-DMSO buffer pH 7.0 when excited at a wavelength of 400 nm. The quenching of fluorescence spectrum of ADG with increasing concentrations of DNA is observed. Quantum yield calculations were made according to the equation as described in (Maiti *et al* 1983).

$$\phi_s = F_s \epsilon_q C_q (0.55) / F_q \epsilon_s C_s \qquad (5)$$

where F is the integrated area of the fluorescence emission curve in arbitrary units, s, the sample and q, the quinine sulphate. A quantitative data of quantum yield at various P/D ratio for various DNA-ADG complex are presented in Table 3. It

Table 3. Quantum yield data for ADG-DNA complexes[a].

DNA/ ADG ratio	CP DNA	CT DNA	EC DNA	ML DNA
20	0.75 ± 0.02	0.70 ± 0.03	0.695 ± 0.02	0.56 ± 0.01
40	0.59 ± 0.01	0.56 ± 0.03	0.55 ± 0.02	0.40 ± 0.03
60	0.51 ± 0.03	0.44 ± 0.02	0.435 ± 0.015	0.30 ± 0.02
80	0.49 ± 0.01	0.43 ± 0.02	0.425 ± 0.02	0.27 ± 0.02
100	0.49 ± 0.05	0.43 ± 0.03	0.425 ± 0.02	0.27 ± 0.02

[a]Five determinations each.

can be seen from the Table 3 that the interaction of ADG with ML DNA is the strongest and with other DNA it varies in the order, EC DNA > CT DNA > CP DNA.

3.5. Thermal stabilization of the ADG-DNA complexes :

ADG enhances the thermal melting temperature (Tm) of all natural DNAs studied. Figure 4 shows the thermal melting profiles of ML DNA and its complex with ADG. Further, the cooperativity of the melting-transition of DNA was unaffected in presence of the alkaloid but a larger hyperchromicity of the ADG-DNA complex as compared to DNA alone was observed which indicated a contribution of the liberated ADG to the overall absorbance at 260 nm (Figure 4). The value of ΔTm (Tm of complex − Tm of native DNA) is significantly dependent on the ADG/DNA ratio, but apparently there is no relation between stabilization and base composition of DNA. Table 4 illustrates the values of ΔTm at the saturation for each complex.

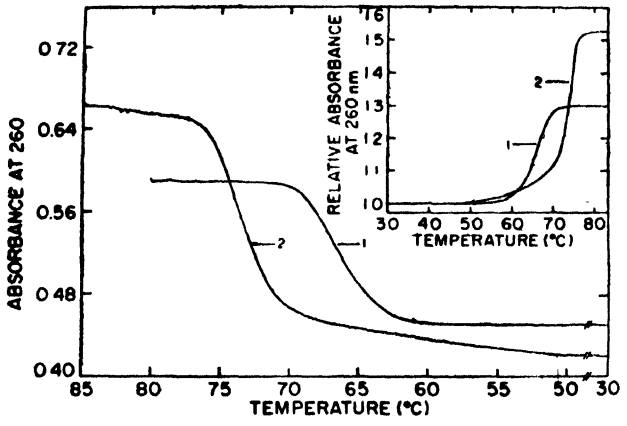

Figure 4. Thermal transition profile of ML DNA 71.7 μM (Curve 1) and in presence of 21.5 μM of ADG (Curve 2) against an appropriately prepared reference sample. Inset: Absorption values of thermal melting transition of ML DNA in presence (Curve 2) and in absence (Curve 1) of ADG at 260 nm relative to that of at 30°C.

Table 4. Observed melting temperature of various DNAs in the presence and absence of ADG in BPE buffer pH 7.0[a].

Polymer	Tm (DNA) (°C)	Tm (complex) (°C)	ΔTm (°C)
CP DNA	49.8	56.0	6.2
CT DNA	56.0	63.0	7.0
EC DNA	61.5	65.0	3.5
ML DNA	68.7	73.5	4.8

[a]Average from three experiments of each DNA.

3.6. CD of the ADG-DNA complexes :

Further evidence to the interaction of the alkaloid to various DNAs is obtained from the CD spectral measurements. In presence of ADG, the B-form CD spectrum of DNA was remarkably perturbed (Figure 5). The intensity of both 270 and 240 nm bands of the spectrum is increased significantly on progressive addition of the alkaloid. The perturbation in the CD spectra of GC-rich ML DNA is greater than that in AT-rich CP DNA. In addition, a rather weak extrinsic CD is seen for the complexes, predominantly positive in character, centred at 315 nm in the wavelength region 300-600 nm (data not shown).

3.7. Viscosity of ADG-DNA complex :

An increase in the viscosity of native rod-like duplex DNA is regarded as a diagnostic feature of intercalation process (Lerman 1961). The viscosity measure-

ments of sonicated rod-like DNA of varying base composition in presence of ADG were performed. The increase in helix length of sheared DNA can be calculated from the experimental results of intrinsic viscosity according to the approximation as described earlier (Maiti *et al* 1982, Maiti and Nandi 1986).

$$L/L_0 = (\eta/\eta_0)^{1/3} = 1 + \beta r \qquad (6)$$

where L, L_0 are the contour length of DNA in presence and absence of the alkaloid and η, η_0 are corresponding values of intrinsic viscosity (approximated by the

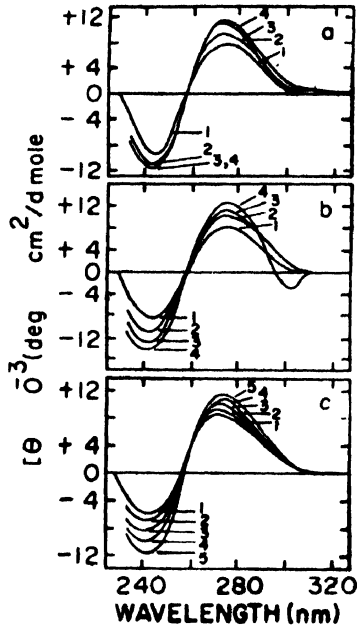

Figure 5. CD spectra of (a) CP DNA (145 μM, Curve 1), (b) EC DNA (150 μM, Curve 1), (c) ML DNA (150 μM, Curve 1) treated with (Curves 2-5) 11.8, 23.6, 35.5, 47.4 μM of ADG respectively.

reduced viscosity of the solution) and β is the slope when L/L_0 is plotted against r. The β value of 2 is predicted for perfect monofunctional intercalators (Lerman 1961). The relative length increase (L/L_0) and the β values for various DNAs are presented in Table 5. This indicates that the enhancement of the helix length for each DNA per alkaloid molecule are comparable to that of an ideal intercalator (Waring 1981a). The β values increases with increasing base composition of DNA.

However, the observation of the viscosity enhancement on binding to linear DNA does not unequivocally establish the actual mode of interaction. Evidence for the intercalation is provided further from the helix unwinding of the covalently closed superhelical DNA (Jones *et al* 1979, King *et al* 1982, Maiti *et al* 1984). It has been observed that ADG induces unwinding-rewinding process on CCS Col

EI DNA like ethidium bromide. Using an unwinding angle of 26° for ethidium, the unwinding angle for ADG was found to be 14°.

Table 5. Viscometric properties of the binding of ADG with various natural DNAs[a].

Polymer	β value	Length enhancement per intercalation site (nm)	Helix length enhancement (%)
CP DNA	1.4	0.29	8.5
CT DNA	1.95	0.33	13.6
EC DNA	1.97	0.335	12.0
ML DNA	2.3	0.39	21.0

[a]Average of three determinations.

4. Discussion

The strong interaction of ADG with DNA was evident from the observation of the typical bathochromic and hypochromic shifts in the absorption spectra, the quenching of fluorescence intensity, causing hindrance to DNA strand separation, perturbations in the CD spectrum, increase in the contour length of sonicated rod-like DNA, induction of the unwinding-rewinding process of covalently closed superhelical DNA, and sign and magnitude of thermodynamic parameters. The bathochromic effect was similar to that observed with intercalated acridines or phenanthridines (Blake and Peacocke 1968, Waring 1965, Zunino *et al* 1972, Maiti *et al* 1982, 1984). Analysis of the absorbance quenching effects indicated that the interaction of calf thymus DNA with ADG led to an intrinsic binding constant of 2.6×10^5 M^{-1} which was of the same order of magnitude as that reported for ideal intercalating agents (Waring 1981a, Maiti *et al* 1982). Binding to DNA showed some dependence on the nucleotide content, as evidenced by the reduction in the intrinsic binding constant for CP DNA which had a lower GC content than ML DNA. The dependence of binding on the GC content of the DNA is also evident from the α-values and from the values of fluorescence quantum yields. The majority of intercalators like sanguinarine (Nandi and Maiti 1985, Maiti *et al* 1982), daunomycin (Chaires *et al* 1982), echinomycin (Waring and Wakelin 1974, Loretto Low *et al* 1984), and others (Wells and Larson 1970, Muller and Crothers 1975, Howe-Grant and Lippard 1979) show GC specificity in DNA binding and even those that do not, such as ethidium (Bresloff and Crothers 1981), may exhibit a CG sequence preference in binding (Kastrup *et al* 1978, Reinhardt and Krugh 1978).

The circular dichroic data reveal that on binding of the alkaloid to any DNA the molar ellipticity of the both 235 and 272 nm bands enhance by about 2 and 1.5 times respectively. Similar enhancement in the CD bands is observed generally when compounds like ethidium or sanguinarine intercalate between DNA base

pairs (Slobodyansky *et al* 1988, Maiti and Nandi 1987b). The CD spectral changes are also sensitive to the base composition of DNA. This again suggests that ADG binds to all DNAs by intercalation. Thus, it has been found that ADG shows a preference for native DNAs whose overall base composition in GC rich, from which we have inferred a preference for GC base pairs as a ADG binding site in natural DNA.

Viscometric studies on sheared rod like linear DNA and covalently closed circular superhelical DNA are well-accepted approaches to test the possibility of and to study both the qualitative and the quantitative aspects of the intercalation of compounds into DNA base pairs (Cohen and Eisenberg 1969, Fox *et al* 1981, Wilson and Jones 1982). An increase in viscosity of a DNA molecule indicates either an increase in axial ratio or rigidity, or both. For thin and rigid molecules like DNA, the major effect is the increase of the axial ratio which reflects, primarily, an increase of the DNA length (Freifelder 1971). The slope β (Table 5) is a parameter related to the functional increase in the contour length of rod-like DNA molecule induced by intercalative ligands. The helix length extension in the case of natural DNAs in presence of ADG can be compared to that obtained for ideal intercalators like ethidium (Waring 1981a, b) and sanguinarine (Maiti *et al* 1982, Nandi and Maiti 1985). The main results is that the increase in contour length of DNA on binding to ADG is higher for GC rich ML DNA than AT-rich CP DNA. These findings are also comparable with the results previously reported for proflavin-DNA (Ramstein *et al* 1972) and sanguinarine-DNA (Nandi and Maiti 1985) complexes where the increase in contour length of duplex DNA depends strongly on its base composition. The binding of ADG to a calf thymus DNA over a wide range of temperature and ionic strengths has been studied in details (Chakraborty *et al* 1990). These results revealed that (i) over the entire range of sodium ion concentration studied, ADG binding process was exothermic, (ii) ADG could exist as a monovalent cationic ligand at neutral pH, (iii) the ADG-calf thymus DNA involved a large favourable non-electrostatic part and (iv) it showed a similar enthalpy-entropy compensatory behaviour like many other intercalators. In the present paper, the temperature dependence of intercalation has been used to derive the enthalpy of ADG binding to the four natural DNAs studied. It has been observed that the binding of the alkaloid is an exothermic process and the binding free energy arises primarily from a large negative enthalpy due to intermolecular interactions at the intercalation-site. With all four DNAs, intercalation is favoured by a negative enthalpic contribution and is opposed by the decrease in entropy. It is interesting to note from the results that the negative enthalpy and entropy change increased with increasing GC content of DNA and also compensated one another to produce a relatively small Gibbs' free energy change. The observed "compensation temperature" (the slope of linear relation of ΔH vs ΔS, 338°K) is significantly higher than the mean harmonic temperature (299°K) indicating that the data are not artifact as proposed by Krug

et al (1976). A linear relation is also observed between Gibbs' free energy change and the enthalpy change which is in favour of this proposal. The thermodynamic parameters obtained presently are enthalpy driven and are comparable to those obtained for daunomycin-CT DNA complex (Chaires 1985). The values of negative enthalpy and entropy changes increase with increasing GC content of DNA which leads to a greater van der Waals stacking interaction between the benzophenanthrine moiety and the adjacent base pairs in GC-rich ML-DNA. These results are in confirmity with the results obtained from spectroscopic and viscometric studies. Thus, it is suggested that enthalpy and entropy compensation may be a general feature of intercalation reaction which also depends on GC content of DNA, but the physical basis of this phenomenon remains unknown.

5. Conclusions

Thus the results strongly indicate that the mode of binding of ADG to DNA is consistent with intercalation model and ADG-DNA complexing has a preference for GC-rich DNA. Intercalation of ADG into four natural DNAs are all characterized by a favourable negative enthalpy and opposed by a negative entropy contribution to binding-site. The process of binding is enthalpy driven. Further exploration will demonstrate the molecular nature of its specificity towards sequences of base pairs in synthetic polynucleotides.

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References

Barcelo F, Martorell J, Gavilanes F and Gonzalez-Ros J M 1988 *Biochem. Pharmacol* **37** 2133

Blake A and Peacocke A R 1968 *Biopolymers* **6** 1225

Bresloff J L and Crothers D M 1981 *Biochemistry* **20** 3547

Chaires J B 1985 *Biochemistry* **24** 403

Chaires J B, Dattagupta N and Crothers D M 1982 *Biochemistry* **21** 3933

Chakraborty S, Nandi R and Maiti M 1990 *Biochem. Pharmacol.* **39** 1181

Chakraborty S, Nandi R, Maiti M, Achari B and Bandyopadhyay S 1989a *Photochem. Photobiol.* **50** 685

Chakraborty S, Nandi R, Maiti M, Achari B, Saha C R and Pakrashi S C 1989b *Biochem. Pharmacol.* **38** 3683

Chaudhuri K, Nandi R and Maiti M 1983 *Indian J. Biochem. Biophys* **20** 488

Chen Z and Zhu D 1987 *The Alkaloids* ed Brossi A, Vol **31** (Orlando, Florida : Academic) p 29

Cohen G and Eisenberg H 1969 *Biopolymers* **8** 45

Crothers D M 1968 *Biopolymers* **8** 575

Debnath D, Kumar G S, Nandi R and Maiti M 1989 *Indian J. Biochem. Biophys.* **26** 201

Fox K R, Harrison N L and Waring M J 1981 *FEBS Lett.* **133** 305

Freifelder D 1971 *J. Mol. Biol.* **60** 401

Howe-Grant M and Lippard S J1979 *Biochemistry* **20** 3547

Jones R L, Davidson M W and Wilson W D 1979 *Biochem. Biophys. Acta* **561** 77
King H D, Wilson W D and Gabbey E J 1982 *Biochemistry* **21** 4982
Kastrup R V, Young M A and Krugh T R 1978 *Biochemistry* **17** 4855
Krug R R, Hunter W G and Grieger R A 1976 *Nature* **261** 566
Lerman L S 1961 *J. Mol. Biol.* **3** 18
Loretto Low C M, Drew H R and Waring M J 1984 *Nucleic Acid Res.* **12** 4865
Maiti M 1986 *Physical Techniques in Biology* ed S N Chatterjee (Calcutta, India : Indian Physical Society) p 52
Maiti M and Chaudhuri K 1981 *Indian J. Biochem. Biophys.* **18** 245
Maiti M and Nandi R 1986 *Indian J. Biochem. Biophys.* **23** 322
——1987a *Anal. Biochem.* **164** 68
——1987b *J. Biomolec. Struct. Dyn.* **5** 159
Maiti M, Nandi R and Chaudhuri K 1982 *FEBS Lett.* **142** 280
——1983 *Photochem. Photobiol.* **38** 245
——1984 *Indian J. Biochem. Biophys.* **21** 158
——1985 *Proc. (Spl. Issue) 5th ISHS Symp.* **1** 103
McGhee J D and Von Hippel 1974 *J. Mol. Biol.* **86** 469
Muller W and Crothers D M 1975 *Eur. J. Biochem.* **54** 267
Nandi R and Maiti M 1985 *Biochem. Pharmacol.* **34** 321
Nandi R, Debnath D and Maiti M 1990 *Biochim. Biophys. Acta* **1049** 339
Nandi R, Chaudhuri K and Maiti M 1985 *Photochem. Photobiol.* **42** 497
Neidle S and Waring M J 1983 *Molecular Aspects of Anticancer Drug Action* (U. K. : MacMillan)
Ramstein J, Dourlent M and Leng M 1972 *Biochem. Biophys. Res. Commun.* **47** 874
Reinhardt C R and Krugh T R 1978 *Biochemistry* **17** 4845
Scatchard G 1949 *Ann. N. Y. Acad Sci.* **51** 660
Slobodyansky E, Stellwagen J and Stellwagen N C 1988 *Biopolymers* **27** 1107
Waring M J 1965 *J. Mol. Biol.* **13** 269
——1981a *Ann. Rev. Biochem.* **50** 159
——1981b *The Molecular Basis of Antibiotic Action* eds E F Gale, E Cundleffe, P E Raynolds, M H Richmond and M J Waring (London : John Weley) p 285
Waring M J and Wakelin L P G 1974 *Nature* **252** 653
Wells R D and Larson J E 1970 *J. Mol. Biol.* **49** 319
Wilson W D 1989 *Nucleic Acids in Chemistry and Biology* eds M Blackburn and M Gait (Oxford, U, K. : IRL Press)
Wilson W D and Jones R L 1982 *Intercalation Chemistry* eds M S Wittengham and A J Jacobson (New York : Academic)
Zimmer C and Wahnert U 1986 *Prog. Biophys. Mol. Biol.* **47** 31
Zunino F, Gambetta R, DiMarco A and Zaccara A 1972 *Biochim. Biophys. Acta* **277** 489